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<b>(21) International Application Number:</b> PCT/US96/05532 <b>(22) International Filing Date:</b> 24 April 1996 (24.04.96)  <b>(30) Priority Data:</b> 08/426,971                      24 April 1995 (24.04.95)                      US  <b>(71)(72) Applicant and Inventor:</b> SHAW, Edward, A. [US/US]; 105 Winterberry Drive, Athens, GA 30606 (US).  <b>(74) Agent:</b> RUDISILL, Stephen, G.; P.O. Box 4433, Houston, TX 77210 (US).		<b>(81) Designated States:</b> AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> REMEDIATION OF PESTICIDE-CONTAMINATED SOIL  <b>(57) Abstract</b>  The present invention provides a method of remediating soil contaminated with organochlorine pesticides. The method comprises introducing methanotrophic bacteria to the soil if methanotrophic bacteria are not present in the soil, and introducing methane and air to the methanotrophic bacteria-containing soil to effect remediation of the soil. An alternative method involves remediation of soil containing the organochlorine pesticide toxaphene using denitrifying bacteria and nitrate.		

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## REMEDIATION OF PESTICIDE-CONTAMINATED SOIL

### FIELD OF THE INVENTION

The present invention is an improved method for the bioremediation of soil contaminated with organochlorine pesticides.

### BACKGROUND OF THE INVENTION

5           Organochlorine pesticides have been used heavily in the U.S. to combat a variety of insect pests. Pesticides have contaminated soils throughout chemical production facilities and from the disposal of container and spray equipment rinsewaters. The United States Environmental Protection Agency has banned most organochlorine pesticides, but not before millions of tons of the pesticides had been  
10       used in various applications across the United states. Most organochlorine pesticides are acutely toxic and mutagenic and carcinogenic under certain conditions. They have been found to be accumulated by animals and plants and are persistent in the environment under normal conditions. For example, the half-life of the organochlorine pesticide toxaphene has been estimated at 11-20 years.

15           Thus, organochlorine pesticides represent a serious threat to the environment and there is clearly a need for innovative technologies for the in-situ remediation of organochlorine pesticide-contaminated soils.

          Excavation and incineration are remedial strategies which have been used because of the complete destruction of the contaminant and liability in a short period  
20       of time. However, they are costly and cause secondary pollution. In-situ and ex-situ chemical treatments involving oxidants, reductants, and alkalis have also been used with varying degrees of success at sites contaminated with organochlorine pesticides.

          It is an object of the invention to provide a method of remediating soil contaminated with organochlorine pesticides. It is a further object of the invention to  
25       provide a method of remediating the contaminated soil which is more cost effective than the existing methods.

### BRIEF DESCRIPTION OF THE FIGURES

          Fig. 1 is a gas chromatogram of toxaphene.

          Figs. 2-4 are gas chromatograms of a sample of soil contaminated with  
30       toxaphene before and after treatment with denitrifying bacteria.

Figs. 5-7 are gas chromatograms of a sample of soil contaminated with toxaphene before and after treatment with methanotrophic bacteria.

Figs. 8-10 are gas chromatograms of a sample of soil contaminated with toxaphene before and after treatment with methanotrophic bacteria.

5 Figs. 11-13 are gas chromatograms of a sample of soil contaminated with toxaphene before and after treatment with methanotrophic bacteria.

Fig. 14-16 are gas chromatograms of a control sample of soil contaminated with toxaphene.

#### SUMMARY OF THE INVENTION

10 The objects of the invention are provided by a method of remediating soil contaminated with organochlorine pesticides. The method comprises the steps of (a) testing the soil for the presence of methanotrophic bacteria; (b) introducing methanotrophic bacteria to the soil if methanotrophic bacteria are not present in the soil; and (c) introducing methane and air to the methanotrophic bacteria-containing  
15 soil to effect remediation of the soil. Methane is present in the methane/air mixture at a concentration of from about 1% to about 8%, preferably about 2% to about 5%.

An alternative method comprises a method for remediating soil containing the organochlorine pesticide, toxaphene. The method comprises the steps of testing the soil for the presence of denitrifying bacteria; (b) introducing denitrifying bacteria to  
20 the soil if denitrifying bacteria are not present in the soil; and (c) introducing nitrate ( $\text{NO}_3^-$ ) to the denitrifying bacteria-containing soil to effect remediation of the soil. Examples of nitrates are potassium nitrate, ammonium nitrate, sodium nitrate, and fertilizer mix containing nitrate salts.

25 Examples of organochlorine pesticides are toxaphene, dieldrin, lindane, aldrin, chlordane, endrin, endrin aldehyde, heptachlor, heptachlor epoxide, and alpha-BHC, beta-BHC, gamma-BHC, delta-BHC, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, endosulfan I, endosulfan II, endosulfan sulfate.

#### DETAILED DESCRIPTION OF THE INVENTION

30 It has been discovered that soils contaminated with organochlorine pesticides may be remediated by methanotrophic or denitrifying bacteria. The present invention is a method of remediating soil contaminated with organochlorine pesticides. The goal is to biotransform the organochloro pesticides to compounds which are more

favorable to aerobic microbial metabolism or degradation, *i.e.*, only oxygen (not methane) would be needed to further degrade the compounds ultimately to carbon dioxide and water.

#### Remediation Using Methanotrophic Bacteria

5           Remediation of soil contaminated with organochlorine pesticides using methanotrophic bacteria is carried out by a method comprising the steps of (a) testing the soil for the presence of methanotrophic bacteria, (b) introducing methanotrophic bacteria to the soil if methanotrophic bacteria are not present in the soil, and (c) introducing methane and air to the methanotrophic bacteria-containing soil to effect  
10 remediation of the soil. Methane is present in the methane/air mixture at a concentration of from about 1% to about 8%, preferably about 2% to about 5%.

Applicants conjecture that remediation of soil contaminated with organochlorine pesticides is carried out by the production by methanotrophic bacteria of the enzyme methane monooxygenase in the presence of methane. Methanotrophic bacteria  
15 under most conditions, depends solely upon methane ( $\text{CH}_4$ ) as a carbon source (electron donor) and molecular oxygen ( $\text{O}_2$ ) as the respiratory compound (electron acceptor). The enzyme methane monooxygenase (MMO) makes methane available as an electron donor. The methane monooxygenase enzyme occurs in two forms, a soluble form and an insoluble form. The soluble form has been demonstrated to have  
20 a low substrate specificity (Stirling et al., Biochem. J., 177, 361-364 (1979). That is, the enzyme will react with 2(cometabolize) a wide range of recalcitrant organic compounds generally unavailable as a primary carbon/energy source to microbes (Stirling et al., 1979). The soluble enzyme becomes active under conditions which are copper or methane stressed (limited). Though not as prevalent, there are  
25 facultative methanotrophs which use other electron donors in addition to methane. This process is aerobic and requires free molecular oxygen as the respiratory compound (electron acceptor).

Methane co-metabolism (usually soluble enzyme-based) has been shown to effectively dechlorinate recalcitrant compounds such as trichloroethylene, dichloro-  
30 ethylene, and vinyl chloride (Oldenhuis et al., Applied and Environmental Microbiology, pp. 2819-2826 (1989); Lanzarone et al., Ground Water, Vol. 28, No.

6, (1990); Roberts et al., Ground Water, Vol. 28, No. 4, (1990); Semprini et al., Ground Water, Vol. 28, No. 5, pp. 715-727 (1990).

### Remediation Using Denitrifying Bacteria

5 Remediation of soil contaminated with the organochlorine pesticide toxaphene may be accomplished using denitrifying bacteria in place of methanotrophic bacteria and introducing nitrate in place of methane and air.

The nitrate is applied to the soil either by laying the solid nitrate onto the soil and subsequently adding water to carry it down into the soil, or by preparing an aqueous solution of the nitrate and applying the solution to the soil. The effective  
10 concentration of nitrate in water may range from about 0.1% to about 10%, preferably from about 0.1 to about 5% , most preferably from about 0.5% to about 1%. Examples of nitrates which may be used are potassium nitrate, ammonium nitrate, sodium nitrate, and fertilizer mix containing nitrate salts.

Remediation of soil using methanotrophic bacteria is expected to work in any  
15 soil which contains both organochlorine pesticides and methanotrophic bacteria. Examples of organochlorine pesticides are toxaphene, dieldrin, lindane, aldrin, chlordane, endrin, endrin aldehyde, heptachlor, heptachlor epoxide, and alpha-BHC, beta-BHC, gamma-BHC, delta-BHC, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, endosulfan I, endosulfan II, endosulfan sulfate. Remediation of soil using denitrifying bacteria is  
20 expected to work in any soil which contains both denitrifying bacteria and toxaphene.

A novel method of practicing the invention comprises driving pipes into the soil to sparge a methane/air mixture into the soil to the desired depth. Previous methods used required first drilling holes into the soil before gases could be pumped into it.

25

### EXAMPLE 1

Determination of Soil Bacterial Content. Toxaphene, a particularly troublesome organochlorine pesticide, is a complex, but reproducible, mixture of chlorinated camphenes sold under names such as Motox, Penphene, Phenacide, Phenatox, Strobane-T, and Toxakil. Toxaphene contains at least 177 C<sub>10</sub> polychloro

derivatives of camphene having the approximate overall empirical formula of  $C_{10}H_8Cl_{10}$ . It is produced by the chlorination of camphene to 67-69 wt. % chlorine and made up of compounds of  $C_{10}H_8Cl_{10}$ ,  $C_{10}H_{18-n}Cl_n$  (mostly polychlorobornanes), and  $C_{10}H_{16-n}Cl_n$  (polychlorobornenes and/or polychlorotri-cyclenes) with  $n = 6$  to  $9$  (The Merck Index, 11th ed. (1989)).

Soil in an area which was used primarily for the production and storage of the organochlorine pesticide, Strobane T-90 (toxaphene), was tested for microbial content. Soil samples were obtained from four locations (A, B, C, and D) at the site where elevated levels of toxaphene contamination had been found during site characterization activities. Extensive aromatic hydrocarbons were released throughout area A and toxaphene concentrations were detected in area A soils at concentrations of 120 mg/kg to 440 mg/kg. Areas B and C have a similar lithology (medium sand) and toxaphene concentrations of 33,000 mg/kg and 41,000 mg/kg, respectively. Soil D was collected from a thin clay layer with a toxaphene concentration of approximately 166,000 mg/kg. Both methanotrophic bacteria and denitrifying bacteria were identified in soils B, C, and D.

### EXAMPLE 2

Degradation of Toxaphene by Denitrification. A 20 foot by 20 foot test plot located within the area tested in Example 1 was initially flooded with 3 to 9 inches of a 10% potassium nitrate solution. The plot was bermed with sand bags and gravel to prevent the nitrate solution from migrating out of the immediate study area.

Four ground-water monitoring points (DENMP-1 through DENMP-4) were installed in the test plot and one recovery well (DEN-MW) was installed immediately downgradient of the study area. The monitoring points were constructed of 1 inch (I.D.) PVC to a depth of approximately three feet, with a six inch section of screen (0.010 inch) and four feet of solid riser. The recovery well was constructed of four inch (I.D.) PVC to an approximate depth of twelve feet, with ten feet of screen (0.010 inch) and 5 feet of solid riser.

Ground-water samples were collected periodically from the recovery well and from the ground-water monitoring points. The samples were analyzed for dissolved chloride ( $Cl^-$ ) and nitrate ( $NO_3^-$ ) using EPA method 300.0. Ground-water samples were also tested in the field for  $NO_3^-$  concentrations with an RQflex hand-held meter



(manufactured by Merck) and Reflectoquant® strips specific for  $\text{NO}_3^-$  (sensitivity range of 3 to 90 mg/L). Whenever  $\text{NO}_3^-$  levels were detected in the recovery well at concentrations above 5 mg/L, the water was pumped through two activated carbon canisters and into a 1,000 gallon polyethylene holding tank. The groundwater was then recirculated through the denitrification test plot until  $\text{NO}_3^-$  concentrations reached appropriate levels.

A ground-water sample was collected from the recovery well on day 81, and analyzed for toxaphene using EPA method 608. At the end of the field pilot study (day 144), another ground-water sample was collected from the recovery well. The sample was split three ways and analyzed for: (1) toxaphene using EPA method 8080, (2) toxaphene by-products using EPA 8270, and (3) apparent toxaphene using a modified EPA method 8080. The modified 8080 method involved a florisil column cleanup procedure intended to remove by-products that might interfere with the toxaphene chromatogram. (Figure 1).

Initial time ( $T_0$ ) soil samples were collected with a decontaminated, stainless steel hand auger on day 1. The sample locations were distributed throughout the test plot at two different depths for each location: A (6" - 12") and B (18" - 24"). The locations were designated L1 through L6.

The samples were analyzed for toxaphene using EPA method 8080. Final time ( $T_f$ ) soil samples were collected on day 144, at approximately the same locations and depths as the  $T_0$  samples. The  $T_f$  samples were split three ways and analyzed for: (1) toxaphene using EPA method 8080, (2) toxaphene by-products using EPA 8270, and (3) apparent toxaphene as described above. A confirmatory soil sample (DEN Middle) was collected at a depth of 6" - 12" on day 224, and analyzed for toxaphene and toxaphene by-products using EPA method 8270. Method 8270 for toxaphene was used because it was suspected that by-products were interfering with the method 8080 toxaphene chromatogram and producing false positive toxaphene concentrations. Chromatograms were retained for each of the sampling events. Figure 1 is the standard chromatogram of toxaphene. The results appear in Table 1 and Figures 2-4.

Analysis of the initial soil sample results using EPA method 8080 (Table 1) indicates that toxaphene concentration in this test plot increased or remained at nearly



the same value throughout the course of the study. The only samples in which a marked decrease in toxaphene was measured ( $\Delta T_0 - T_c$ ) was in L1-A and L4-A. Analysis of the change in toxaphene concentrations between the final soil analysis and the final soil analysis after the florisil column cleanup ( $\Delta T_f - T_c$ ) indicates a decrease in toxaphene concentrations in many cases, suggesting that other compounds interfered with the method and yielded false positive data. When a confirmation sample (DEN middle) was collected from the center of the plot at a depth of 6''-12'' and analyzed for toxaphene using EPA method 8270, toxaphene concentrations were below detectable levels (Table 9). Chloride ion ( $Cl^-$ ) concentrations measured in the monitoring points increased slightly after 30 days (Table 6), but then fell to levels below initial concentrations by the end of the study. Analysis of the ground-water sample results from the downgradient monitoring well (Table 5) shows that dissolved phase toxaphene concentrations nearly doubled during the study and that inferring compounds caused some false positive data.

15

### EXAMPLE 3

Degradation of Toxaphene by Methanotrophic Bacteria. A 20 foot by 20 foot test plot located within the area tested in Example 1 and approximately 60 feet due west of the denitrification test plot used in Example 2 was used to test for the degradation of toxaphene by methanotrophic bacteria. As for the denitrification plot, high toxaphene concentrations were the criterion for the placement of the pilot study area. A 5% methane/air mixture was delivered to the toxaphene impacted soils by using five foot deep injection points constructed of 0.75 inch (I.D.) black pipe. The injection pressure of the methane/air mixture was regulated so that the average injection rate per point was 0.5 SCFM.

25

Five methane/air injection points were installed in the upgradient portion of the plot. An "aerobic curtain" was created in the downgradient portion of the plot by installing five air-only injection points. The purpose of the air-only points was to introduce oxygen to the shallow groundwater to promote the aerobic degradation of any toxaphene by-products before they migrated out of the plot.

30

The methane cometabolism pilot study plot was constructed in a fashion similar to that of the denitrification test plot, including a sand bag and gravel berm. Also, four ground-water monitoring points (METHMP-1 through METHMP-4) were

installed in the study area, and one recovery well (METH-MW) was located immediately downgradient of the study area. Ground-water samples were collected periodically from the recovery well and from the monitoring points in the plot. The samples were analyzed for dissolved chloride ( $\text{Cl}^-$ ) and nitrate ( $\text{NO}_3^-$ ) using EPA  
5 method 300.0.

A ground-water sample was collected from the recovery well on day 81, and analyzed for toxaphene using EPA method 608. At the end of the field pilot study (day 144), another ground-water sample was collected from the recovery well. The sample was split three ways and analyzed for: (1) toxaphene using EPA method  
10 8080, (2) toxaphene by-products using EPA 8270, and (3) apparent toxaphene as previously defined.

Initial time ( $T_0$ ) soil samples were collected with a decontaminated, stainless steel hand auger on day 1. The sample locations were distributed throughout the test plot at two different depths for each location: A (6" - 12") and B (18" - 24"). The  
15 locations were designated L7 through L12.

The samples were analyzed for toxaphene using EPA method 8080. Final time ( $T_f$ ) soil samples were collected on day 144, at approximately the same locations and depths as the  $T_0$  samples. The  $T_f$  samples were split three ways and analyzed for: (1) toxaphene using EPA method 8080, (2) toxaphene by-products using EPA  
20 8270, and (3) apparent toxaphene using the method previously discussed. Confirmatory soil samples were collected on day 224, and analyzed for toxaphene and toxaphene by-products using EPA method 8270. One sample was collected near the methane/air injection points (METH NE Corner) and one was obtained near the aerobic curtain (METH Aerobic Zone). Chromatograms were retained for each of  
25 the sampling events. The results appear in Table 2 and Figures 5-13.

Analysis of the initial soil sample results from this plot using EPA method 8080 (Table 2) indicates that toxaphene concentrations increased in some locations of the plot and decreased in other locations. A marked decrease in toxaphene was measured ( $\Delta T_0 - T_c$ ) in sample L8-A ( $> 90,000 \mu\text{g/kg}$ ). Analysis of the change in  
30 toxaphene concentrations between the final soil analysis and the final soil analysis after the florisil column cleanup ( $\Delta T_f - T_c$ ) indicates a decrease in toxaphene concentrations in every sample but L7-A. These data suggest that other compounds

interfered with the method and yielded false positive data. When confirmation samples (METH NE Corner and METH aerobic zone) were collected from the plot and analyzed for toxaphene using EPA method 8270, toxaphene concentrations were below detectable levels (Table 4). Chloride ion ( $\text{Cl}^-$ ) concentrations measured in the monitoring points were higher initially than the denitrification plot and nearly doubled in METHMP-1 and METHMP-2 after approximately 30 days (Table 7). Analysis of the ground-water sample results from the downgradient monitoring well shows that toxaphene concentrations were over an order of magnitude less at the end of the study than at the beginning (Table 5).

10

#### EXAMPLE 4

Controlled Experiment. A 20 foot by 20 foot square control plot was placed near the pilot study areas used in Examples 2 and 3. The control plot was not bermed and did not have any ground-water monitoring points, injection points, or a downgradient recovery well. The initial intention was to leave this plot undisturbed throughout the study.

15

Initial time ( $T_0$ ) soil samples were collected with a decontaminated, stainless steel hand auger on day 1. The sample locations were distributed throughout the test plot at two different depths for each location: A (6" - 12") and B (18" - 24"). The locations were designated C1 and C2.

20

The samples were put on ice and shipped to the laboratory under chain of custody. The soils were then analyzed for toxaphene by EPA method 8080. Final time ( $T_f$ ) soil samples were collected on day 144, at approximately the same locations and same depths as the  $T_0$  samples.

The  $T_f$  samples were analyzed using the same methods discussed above for the denitrification and methane cometabolism plots. A confirmatory control sample was collected on day 224, in an area upgradient from the control plot and analyzed for toxaphene and toxaphene by-products using EPA method 8270. Chromatograms were retained for each of the sampling events. Results appear in Table 3 and Figures 14-16.

30

Analysis of the initial soil sample results from the control plot area using EPA method 8080 (Table 3) indicates that toxaphene concentrations increased in every

sample but C2-B (Table 3). The data also show that interfering compounds were present in the control plot soil samples. When a confirmation sample (TOX test) was obtained from an area upgradient of the control plot and analyzed for toxaphene using EPA method 8270, toxaphene was below detectable levels (Table 4).

5

#### EXAMPLE 5

The confirmatory soil samples collected in Examples 2, 3, and 4 were analyzed for toxaphene and toxaphene by-products using EPA method 8270. Sample "DEN Middle" was collected at a depth of 6" - 12" in the denitrification test plot of Example 2. Additional samples were taken near the methane/air injection points ("METH NE Corner") and near the aerobic curtain ("METH Aerobic Zone") in the methane cometabolism test plot of Example 3. Sample "TOX" was taken in an area upgradient from the control test plot of Example 4. Results appear in Table 4.

10

#### EXAMPLE 6

The ground water monitored in the test plots of Examples 2, 3, and 4 was analyzed. The results in Table 5 show a significant decrease in the toxaphene levels in the methane cometabolism test plot (Example 3). The increase in toxaphene concentration seen the denitrification test plot (Example 2) may have been due to increased solubility of the degraded toxaphene compounds.

15

#### EXAMPLE 7

Chloride concentrations were measured in the denitrification test plots of Examples 2 and 3. Results appear in Tables 6 and 7.

20

#### EXAMPLE 8

The confirmatory soil samples taken in Examples 2, 3, and 4 were analyzed for chemical content. Compounds that were tentatively identified as being present are listed in Table 9. Sample IDs 1-5 in Table 9 refer to the following: Sample ID 1 = METH NE Corner 6-12"; Sample ID 2 = METH NE Corner 18-24"; Sample ID 3 = METH Aerobic Zone; Sample ID 4 = DEN Middle; and Sample ID 5 = TOX Control Test.

25

#### Discussion of Toxaphene Degradation

30

Because the presence of interfering compounds caused false positive data when quantifying toxaphene with EPA method 8080, the best data for measuring actual toxaphene degradation during these field trials is the chromatograms. The toxaphene

chromatogram is a complex mixture of many peaks with a wide range of GC retention times (Figure 1). As the toxaphene molecule degrades the chromatographic fingerprint may be altered in several ways. As the fraction of less-chlorinated toxaphene components/by-products increases (compounds that have shorter GC retention times), there will be a shift in the chromatogram to the left. An increased concentration of interfering by-products will also result in an elevated baseline. Analysis of the chromatogram before and after a cleanup procedure (i.e., silica gel or florisil) also provides evidence that degradation occurred and that interfering by-products were removed with the cleanup.

10           Analysis of the toxaphene chromatogram (EPA method 8080) from sample L1-B illustrates the overall patterns observed in the denitrification plot. Chromatograms from this plot exhibited relatively few alterations in the toxaphene fingerprint as compared to the toxaphene standard chromatogram (Figures 1-4). This is in contrast to the weathered/shifted chromatogram examples from the methane cometabolism plot (Figures 5-13). Note that the example chromatograms from the methane cometabolism plot exhibit the following characteristics: (1) an increase in the number and size of peaks having shorter GC retention times; (2) an elevated baseline between initial ( $T_0$ ) and final samples ( $T_f$ ); and (3) the cleanup procedure removed many of the interfering compounds.

20           When the confirmation soil samples were analyzed for toxaphene and toxaphene by-products using EPA method 8270 (GC/MS method), several tentatively identified interfering compounds/by-products were identified (Table 8) with retention times ranging from 2.7 to 9.2 minutes. These compounds appear to the left of the standard toxaphene fingerprint and have solubility values greater than toxaphene.

25           Note that in the control test plot the shift between the initial time and final time toxaphene concentrations was only 5% (Table 3). These data indicate that the control plot conditions were in fact well controlled. The good control in this plot is confirmed by chromatographic evidence from sample C1-B (Figures 14-16). There were few alterations in the toxaphene fingerprint from initial time ( $T_0$ ) to final time  
30           ( $T_f$ ) and the chromatograms reflected the characteristic pattern of the technical toxaphene example chromatogram (Figure 1).

Based on the chromatographic evidence, the toxaphene molecule did appear to be degraded/biotransformed in the denitrification plot. However, analysis of the chromatograms from the toxaphene-contaminated soils treated in the methane cometabolism plot indicates a much greater extent of degradation. The denitrification plot failure is only an apparent failure due to a function of hydrostratigraphy.

Our ability to accurately quantify toxaphene in the soils during this field pilot study was limited by EPA method 8080. While this method is designed for determining the concentration of technical grade or relatively unweathered toxaphene, it does not seem to be appropriate for quantifying degraded/weathered toxaphene concentrations. The presence of more soluble by-products in the sample and compounds that interfere with the chromatogram can yield false positive toxaphene concentrations. Even the florisil column cleanup procedure did not remove all of the interference. Therefore, increases in apparent toxaphene concentrations as reported in the tables are not an indication that the remediation method failed or was ineffective. The ineffectiveness of the remediation method is more appropriately evaluated by changes in the chromatograms, especially the changes in the chromatograms after the florisil column cleanup procedure. Preliminary results indicate that a GC/MS method, like EPA 8270 or 8260 may be better suited for quantifying degraded toxaphene concentrations in the field.

Analysis of the chromatograms, especially those from the methane cometabolism plot that show significant alterations between initial and final time samples, indicates that the soils no longer contain technical toxaphene as regulated. Instead, we are dealing with degraded/weathered toxaphene fractions that are more susceptible to aerobic microbial degradation. Interference from by-products can also occur right in the middle of the chromatogram. The chromatogram does not necessarily have to be shifted left to show degradation.



TABLE 1

## Denitrification Pilot Study Soil Samples

Toxaphene Concentration ( $\mu\text{g/kg}$ )						
Sample ID	Initial ( $T_0$ )	Final ( $T_f$ )	After Florisil Cleanup ( $T_c$ )	$\Delta T_0 - T_f$	$\Delta T_f - T_c$	$\Delta T_0 - T_c$
L1-A	190,000	18,000	15,000	172,000	3000	175,000
L1-B	230,000	320,000	190,000	-90,000	130,000	40,000
L2-A	310,000	530,000	540,000	-220,000	-10,000	-230,000
L2-B	190,000	230,000	220,000	-40,000	10,000	-30,000
L3-A	91,000	230,000	240,000	-139,000	-10,000	-149,000
L3-B	280,000	280,000	230,000	0	50,000	50,000
L4-A	220,000	47,000	34,000	173,000	13,000	186,000
L4-B	40,000	90,000	66,000	-50,000	24,000	-26,000
L5-A	75,000	190,000	200,000	-115,000	-10,000	-125,000
L5-B	31,000	40,000	37,000	-9,000	3,000	-6,000
L6-A	13,000	35,000	30,000	-22,000	5,000	-17,000
L6-B	95,000	140,000	160,000	-45,000	-20,000	-65,000



TABLE 2  
Methane Cometabolism Pilot Study Soil Samples

		Toxaphene Concentration ( $\mu\text{g/kg}$ )					
Sample ID		Initial ( $T_0$ )	Final ( $T_f$ )	After Florisil Cleanup ( $T_c$ )	$\Delta T_0 - T_f$	$\Delta T_f - T_c$	$\Delta T_0 - T_c$
5	L7-A	BDL	15,000	22,000	-15,000	-7,000	-22,000
	L7-B	11,000	35,000	35,000	-24,000	0	-24,000
	L8-A	130,000	53,000	35,000	77,000	18,000	95,000
	L8-B	40,000	48,000	28,000	-8,000	20,000	12,000
10	L9-A	43,000	41,000	31,000	2,000	10,000	-12,000
	L9-B	BDL	5,900	3,500	-5,900	2,400	-3,500
	L10-A	76,000	130,000	110,000	-54,000	20,000	-34,000
	L10-B	75,000	73,000	56,000	2,000	17,000	19,000
15	L11-A	4,500	41,000	35,000	-36,500	6,000	-30,500
	L11-B	BDL	BDL	BDL	0	0	0
	L12-A	9,700	43,000	33,000	-33,300	10,000	-23,300
	L12-B	BDL	21,000	16,000	-21,000	5,000	-16,000

TABLE 3  
Control Plot Soil Samples

Sample ID	Toxaphene Concentration ( $\mu\text{g}/\text{kg}$ )			$\Delta$ $T_0 - T_f$	$\Delta$ $T_f - T_c$	$\Delta$ $T_0 - T_c$
	Initial ( $T_0$ )	Final ( $T_f$ )	After Florisil Cleanup ( $T_c$ )			
C1-A	62,000	320,000	200,000	-258,000	120,000	-138,000
C1-B	150,000	270,000	210,000	-120,000	60,000	-60,000
C2-A	200,000	1,300,000	1,200,000	$-1.1 \times 10^6$	100,000	$-1.0 \times 10^6$
C2-B	3,100,000	1,900,000	1,300,000	$1.2 \times 10^6$	$1.77 \times 10^6$	$2.97 \times 10^6$

TABLE 4  
Confirmatory Soil Samples Collected

Sample ID	Toxaphene Concentration
METH NE Corner 6-12"	ND
METH NE Corner 18-24"	ND
DEN Middle	ND
TOX Control Test	ND

ND = not detected

TABLE 5  
Ground-Water Samples from Denitrification and Methane Cometabolism Recovery Wells

Example	Toxaphene Concentration ( $\mu\text{g}/\text{L}$ )			$\Delta$ $T_0 - T_f$	$\Delta$ $T_f - T_c$	$\Delta$
	Initial ( $T_0$ )	Final ( $T_f$ )	After Florisil Cleanup ( $T_c$ )			
2	1,300	2,300	2,100	-1,000	200	-800
3	31	7.0	2.6	24	4.4	28.4

TABLE 6  
Chloride (Cl<sup>-</sup>) Concentrations in Denitrification Test Plot  
(reported in mg/L)

5

Sample ID	Day				
	30	44	58	85	143
DENMP-1	24.1	19	61.5	24.2	23
DENMP-2	25.3	35.1	--	--	13
DENMP-3	23.8	18	57.5	16.1	15
DENMP-4	20.1	15.7	51.5	21	15
DEN-MW	--	66.6	--	--	42

10

TABLE 7  
Chloride (C<sup>-</sup>) Concentrations in Methane Cometabolism Test Plot  
(reported in mg/L)

15

Sample ID						
	30	44	58	85	143	224 (before/ after purging)
METHMP-1	49.9	141.5	82.3	82.41	150	64/71
METHMP-2	286.3	408.6	455.9	425.1	195	41/300
METHMP-3	76.7	84.4	78.2	--	69	--
METHMP-4	58.2	54.9	101.6	76.6	20	--
METH-MW	--	25.6	--	--	38	--

TABLE 8  
Tentatively Identified Compounds (TIC) in Confirmatory Soil Samples  
Summary of Laboratory Results Using EPA Method 8270

	Compound Name	CAS Number	Average Retention Time (min)	Estimated Concentration (mg/kg)				
				Sample ID				
				1	2	3	4	5
5	2-Pentanone, 4-hydroxy-4-methyl-	123-42-2	2.655	9.6	8.7	11	11	0
	Tricyclo {2.2.1.0 <sup>2,6</sup> } heptane, 1,7,7-trimethyl-	508-32-7	3.245	0	0	10	0	21
	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-	80-56-8	3.334	16	16	33	0	26
10	1,3,6-Heptatriene, 2,5,5-trimethyl-	29548-02-5	3.447	0	0	59	0	69
	Benzene, 1-chloro-2-methyl-	95-49-8	3.464	38	35	0	0	0
	Benzene, 1-chloro-4-methyl-	106-43-4	3.491	36	19	18	0	21
	Benzene, 1-ethyl-2-methyl-	611-14-3	3.511	0	0	0	11	0
	Benzene, 1,3,5-trimethyl-	108-67-8	3.559	0	0	0	4.1	0
15	Cyclohexane, 3-methyl-6-(1-methylethyl)-, trans	1124-26-1	3.786	0	0	13	0	0
	1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)-	99-83-2	3.821	0	0	9.4	0	0
20	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	99-86-5	3.916	0	0	26	0	0
	1-Hexanol, 2-ethyl-	104-76-7	3.937	26	32	0	0	32
	Benzene, 1-methyl-4-(1-methylethyl)-	99-87-6	3.987	0	0	180	0	0
	Phenol, 2-methyl-	95-48-7	4.104	0	6.8	0	0	0
	Benzene, 1,2-dichloro-3-methyl-	32768-54-0	4.650	120	76	190	110	68
25	Benzene, 1,2-dichloro-4-methyl-	95-75-0	4.803	0	0	0	0	71
	Benzene, 1,3-dichloro-2-methyl-	118-69-4	4.851	0	0	0	130	0
	Benzene, 1,2,4-trichloro-3-methyl-	2077-46-5	5.613	16	0	9.8	5.4	0
	Benzene, 1,2,3,5-tetrachloro-	634-90-2	6.213	0	0	0	0	32
	Benzene methanol, 2,4-dichloro-	1777-82-8	6.346	0	0	16	0	0
30	Benzene, 1,1'-(1,2-ethenediyl) bis-	103-29-7	6.960	0	10	0	0	0
	Benzene, 1-methyl-2-(phenylmethyl)-	713-36-0	7.010	0	15	0	0	15
	Benzene, 1-methyl-4-(phenylmethyl)-	620-83-7	7.037	0	16	0	0	12

5

Compound Name	CAS Number	Average Retention Time (min)	Estimated Concentration (mg/kg)				
			Sample ID				
Hexanoic acid, 2-ethyl-, 2-ethylhexyl ester	7425-14-1	7.280	0	0	0	0	27
Benzene, 1,1'-(1,2-ethenediyl), (E)	103-30-0	7.897	8.3	9.2	0	0	0
Benzene, 1,1'-ethenylidenebis-	530-48-3	8.832	8.1	0	0	0	0
Benzeneethanol, $\beta$ -methyl- $\beta$ -phenyl-	74421-26-4	9.829	8.4	8.9	0	0	0
Octane, 2-bromo-	557-35-7	9.235	0	0	0	0	31

What is claimed is:

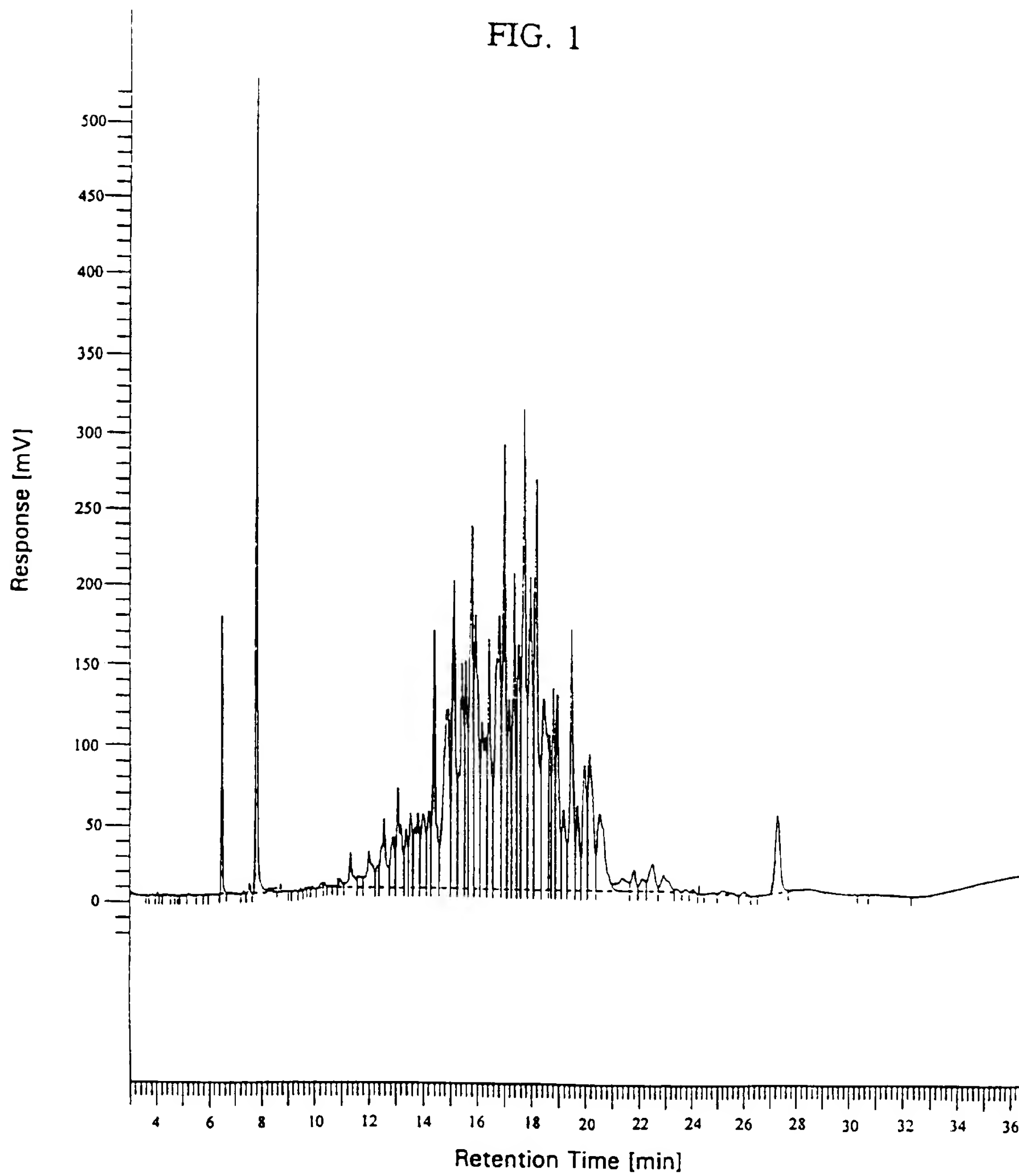
1. A method of remediating methanotrophic bacteria-containing soil contaminated with organochlorine pesticides, comprising introducing methane and air to the soil.
2. The method of claim 1 wherein the organochlorine pesticide is toxaphene, dieldrin, lindane, aldrin, chlordane, endrin, endrin aldehyde, heptachlor, heptachlor epoxide, and alpha-BHC, beta-BHC, gamma-BHC, delta-BHC, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, endosulfan I, endosulfan II, endosulfan sulfate.
3. The method of claim 2 wherein the organochlorine pesticide is toxaphene.
4. The method of claim 1 wherein the concentration of methane in air is in the range of from about 1% to about 8%.
5. The method of claim 4 wherein the concentration of methane in air is in the range of from about 2% to about 5%.
6. A method of remediating methanotrophic bacteria-free soil contaminated with organochlorine pesticides, comprising the steps of:
  - (a) introducing methanotrophic bacteria to the soil; and
  - (b) introducing methane and air to the methanotrophic bacteria-containing soil.
7. The method of claim 6 wherein the organochlorine pesticide is toxaphene, dieldrin, lindane, aldrin, chlordane, endrin, endrin aldehyde, heptachlor, heptachlor epoxide, and alpha-BHC, beta-BHC, gamma-BHC, delta-BHC, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, endosulfan I, endosulfan II, endosulfan sulfate.
8. The method of claim 7 wherein the organochlorine pesticide is toxaphene.
9. The method of claim 6 wherein the concentration of methane in air is in the range of from about 1% to about 8%.
10. The method of claim 9 wherein the concentration of methane in air is in the range of from about 2% to about 5%.
11. A method of remediating denitrifying bacteria-containing soil contaminated with toxaphene, comprising introducing nitrate and water to the denitrifying bacteria-containing soil.
12. The method of claim 11 wherein the nitrate is potassium nitrate, ammonium nitrate, sodium nitrate, or fertilizer mix containing nitrate salts.

13. A method of remediating denitrifying bacteria-free soil contaminated with toxaphene, comprising the steps of:
  - (a) introducing denitrifying bacteria to the soil; and
  - (b) introducing nitrate and water to the denitrifying bacteria-containing soil.
14. The method of claim 13 wherein the nitrate is potassium nitrate, ammonium nitrate, sodium nitrate, or fertilizer mix containing nitrate salts.



1/16

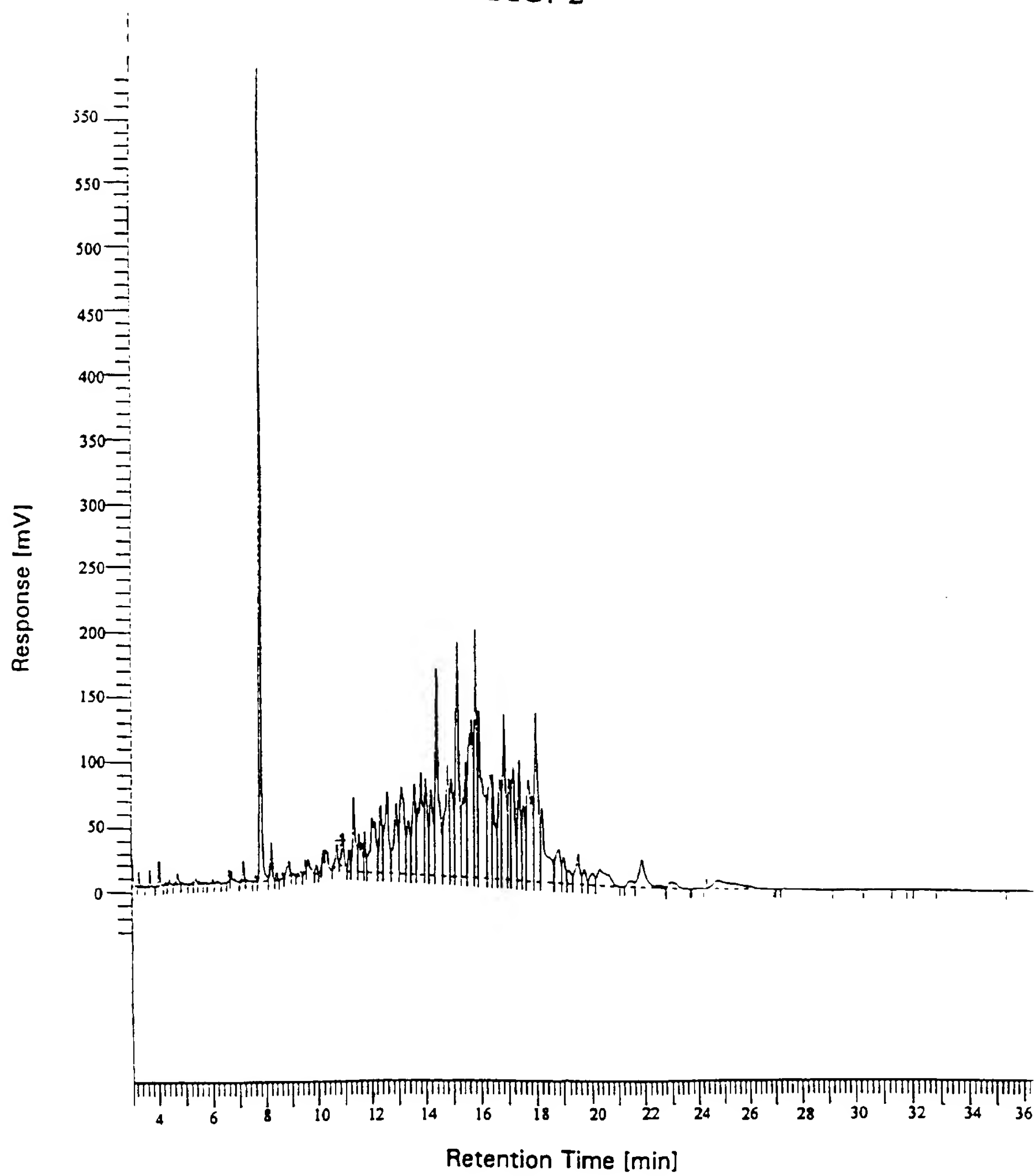
FIG. 1



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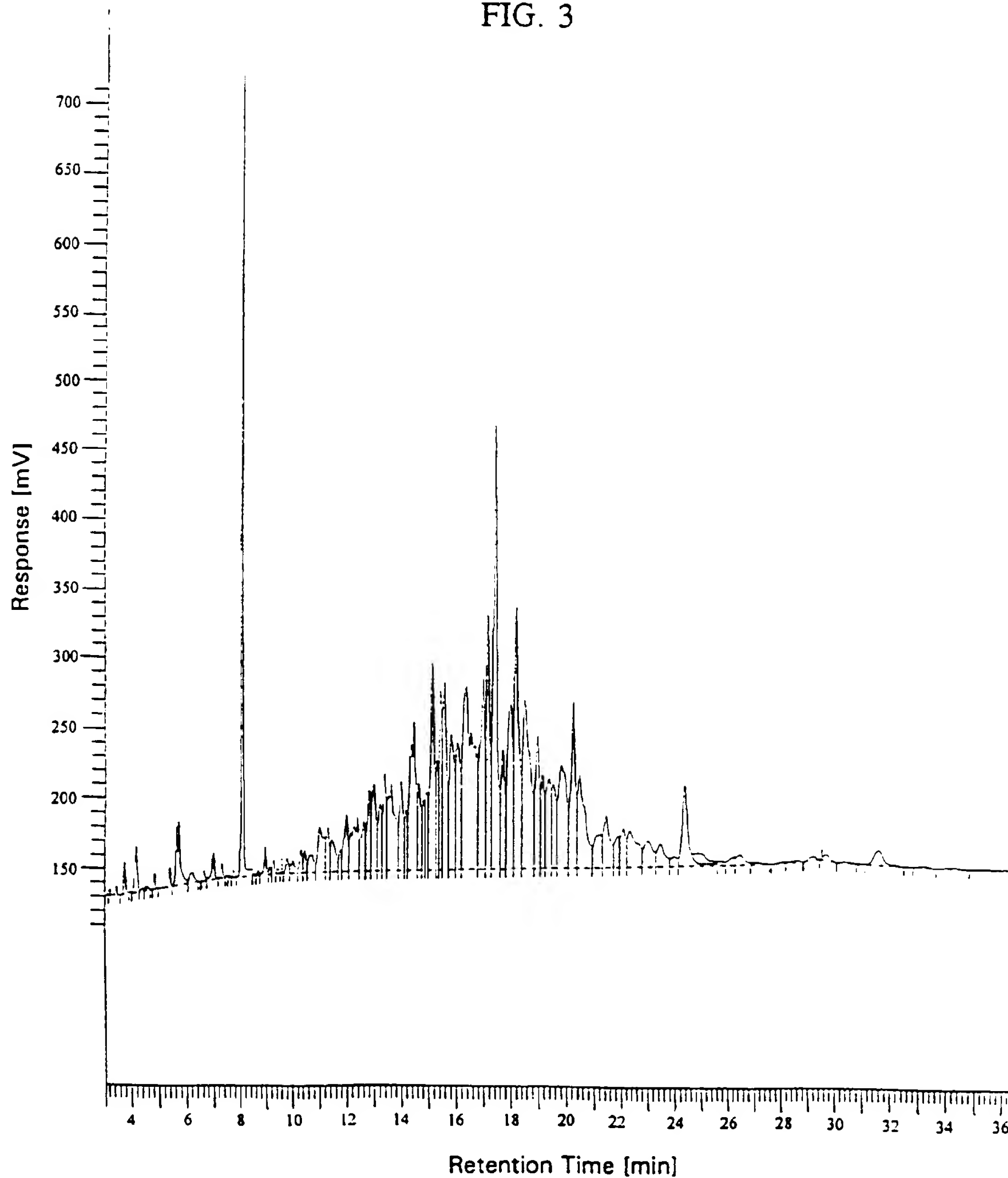
2/16

FIG. 2



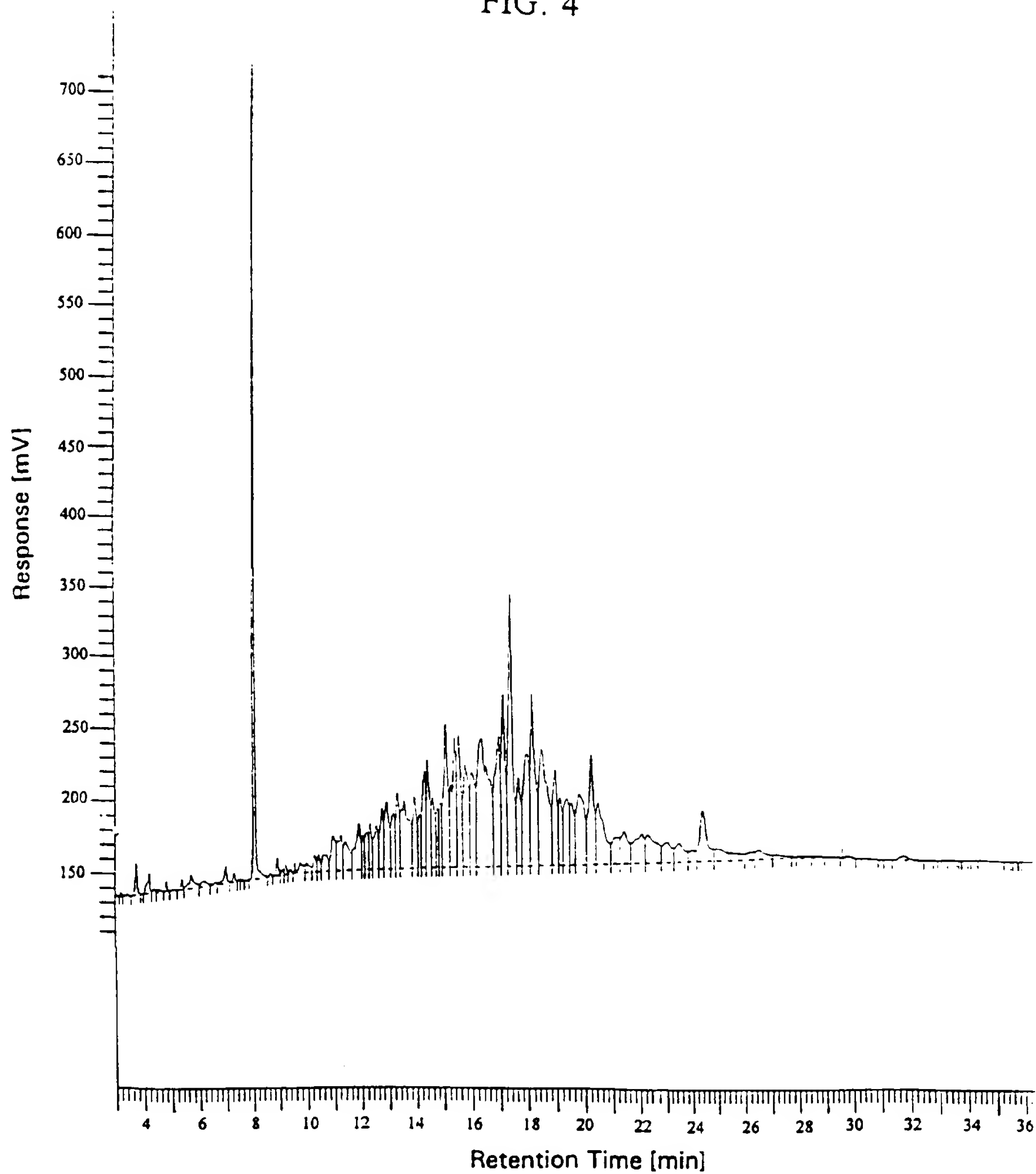
3/16

FIG. 3



4/16

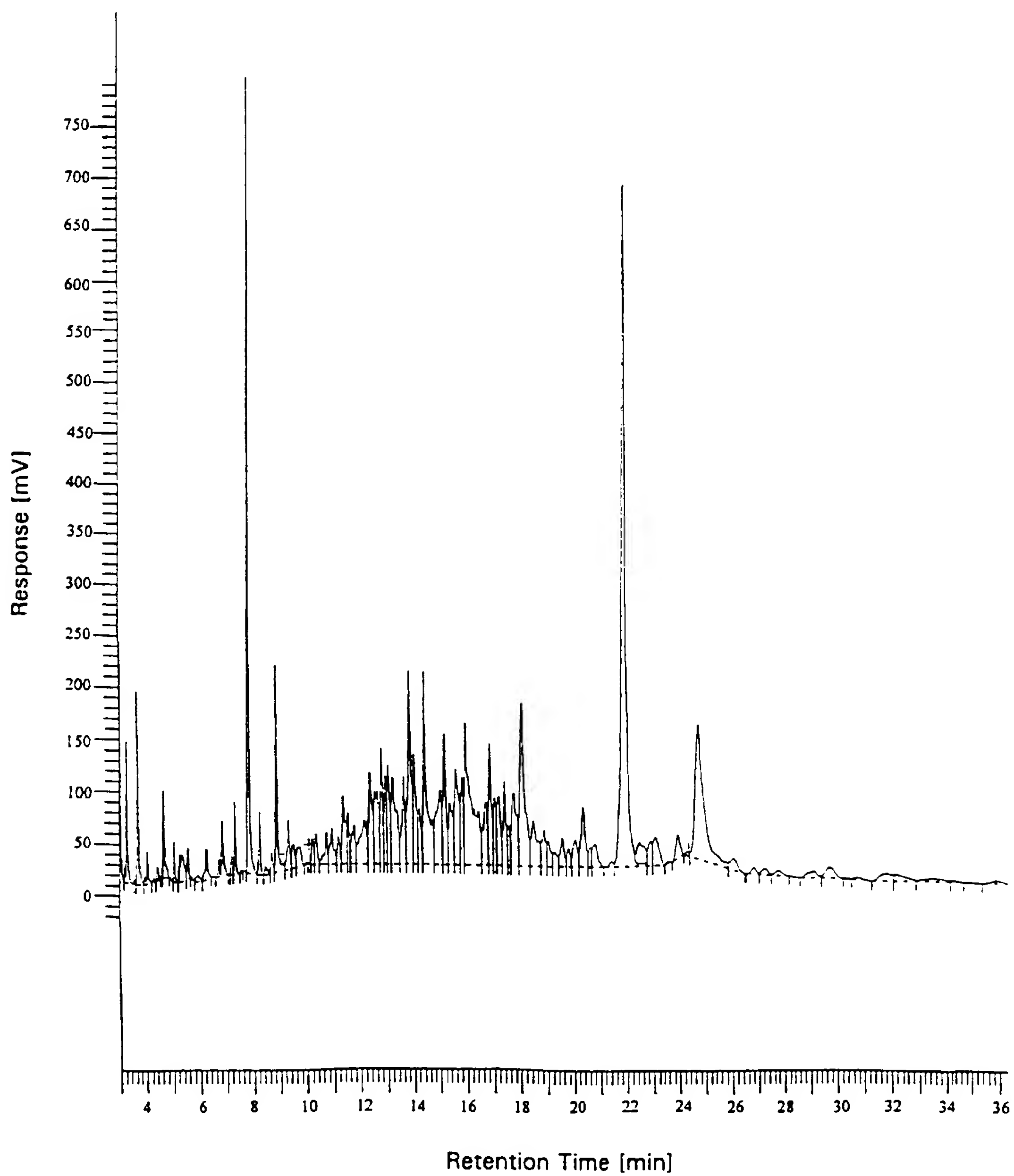
FIG. 4



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5/16

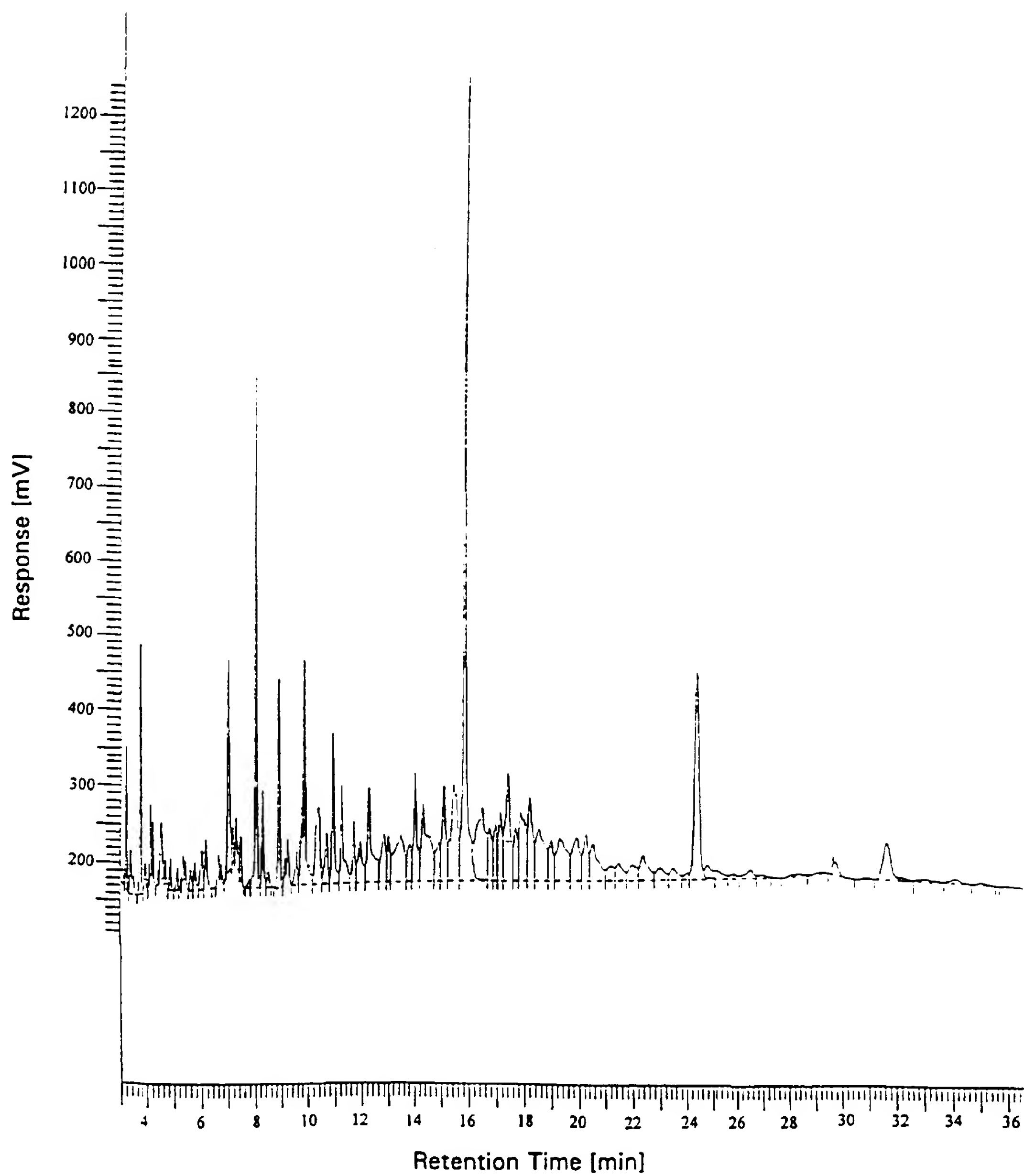
FIG. 5



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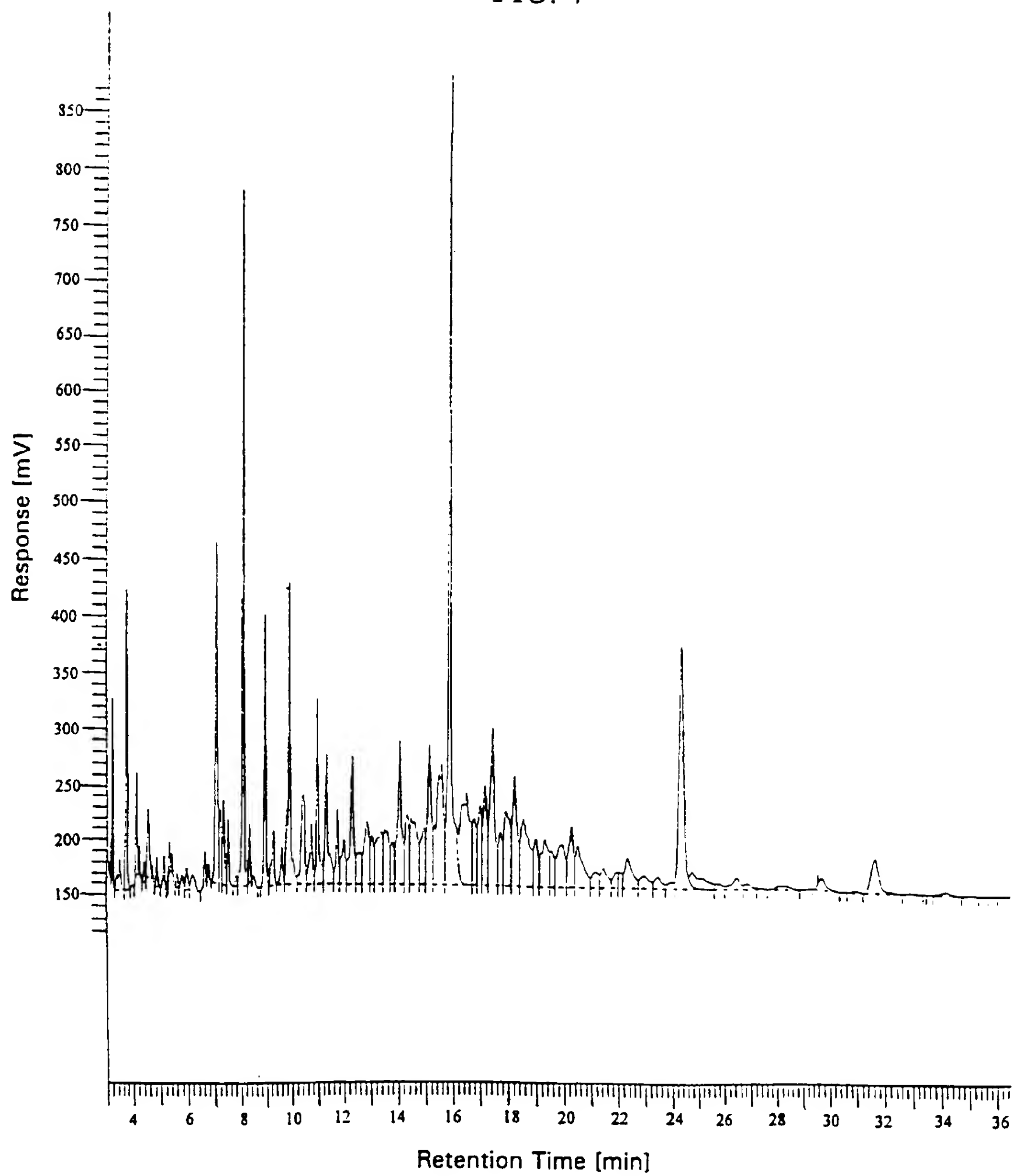
6/16

FIG. 6



7/16

FIG. 7

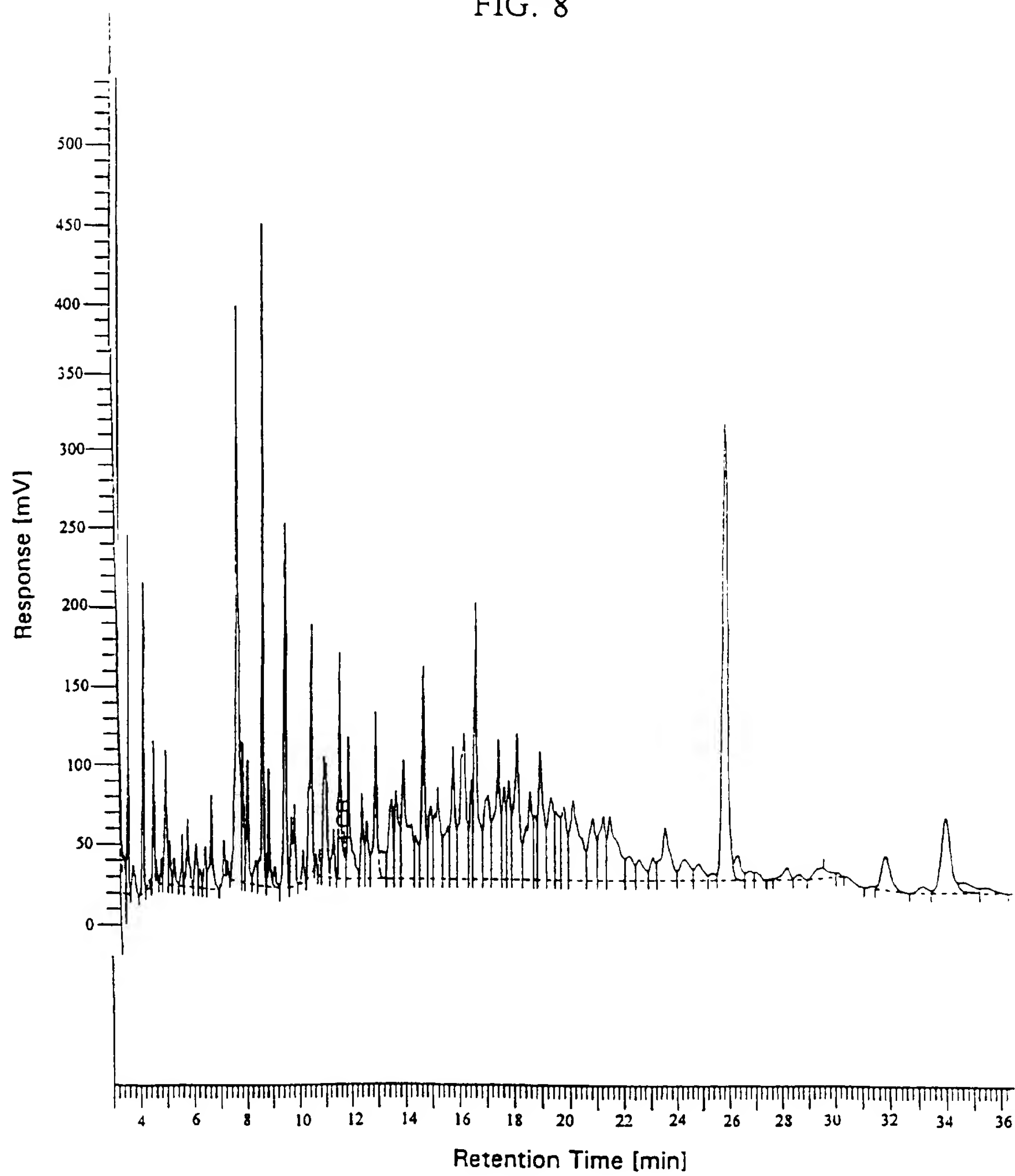


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8/16

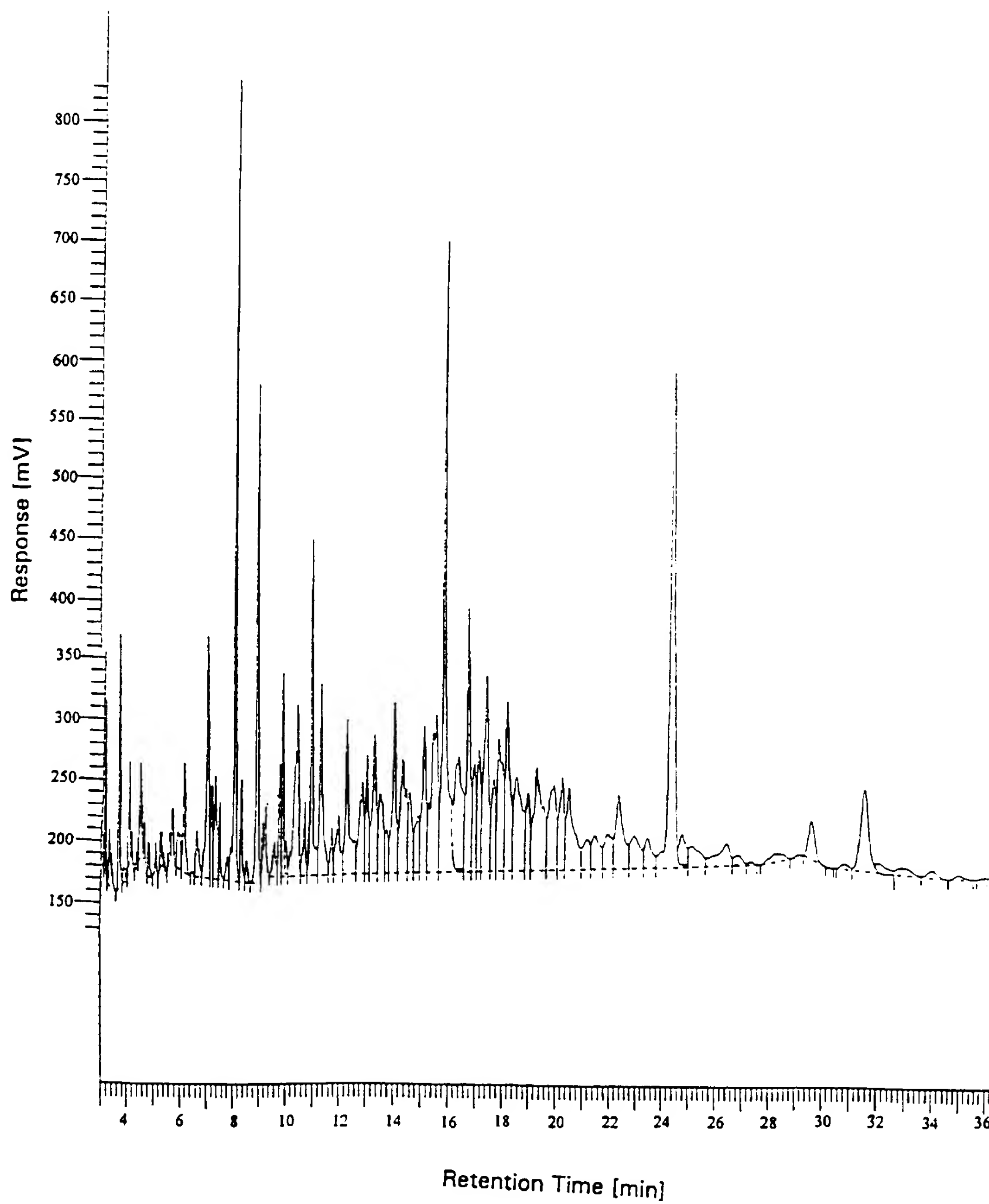
FIG. 8



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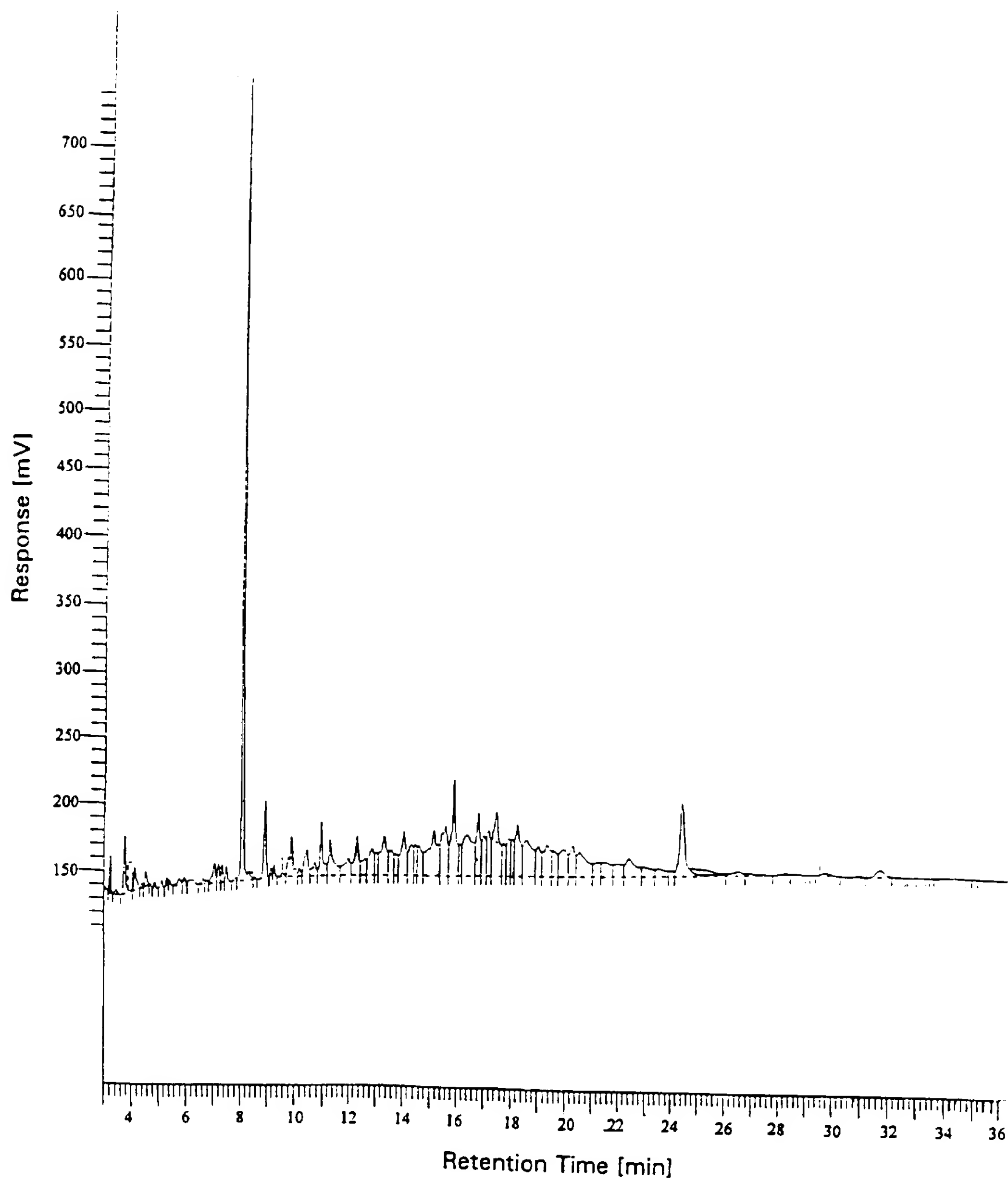
9/16

FIG. 9



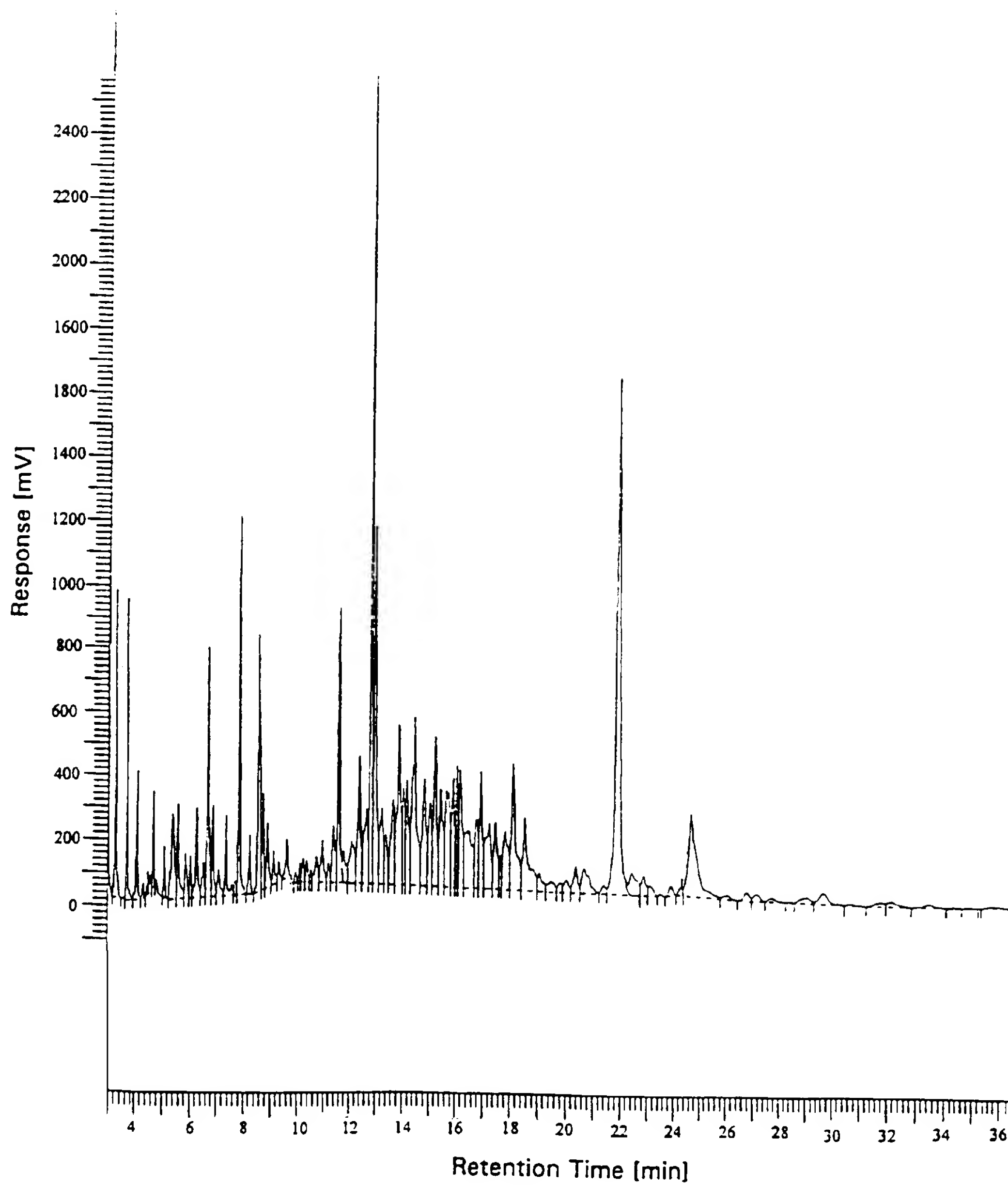
10/16

FIG. 10



11/16

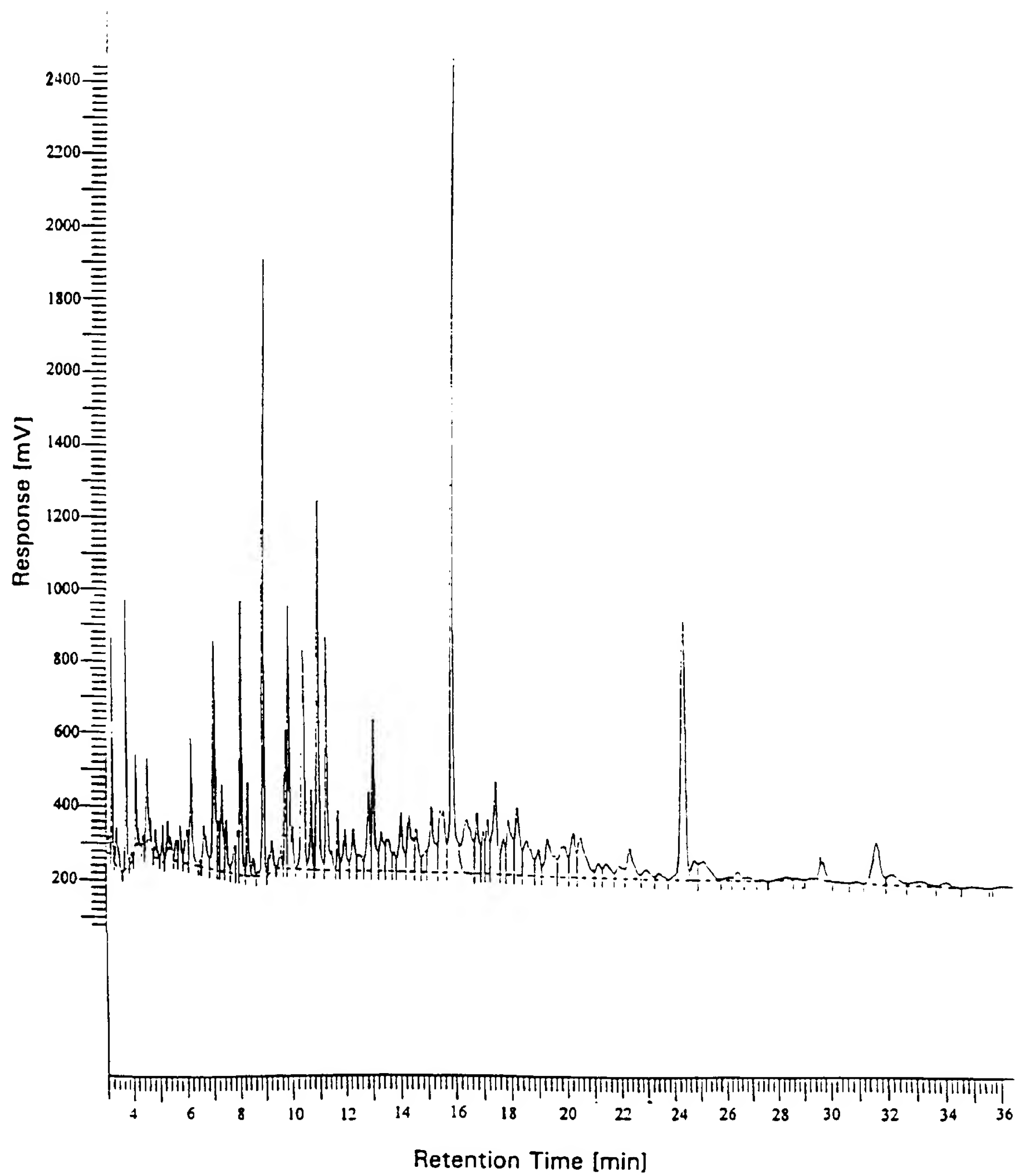
FIG. 11



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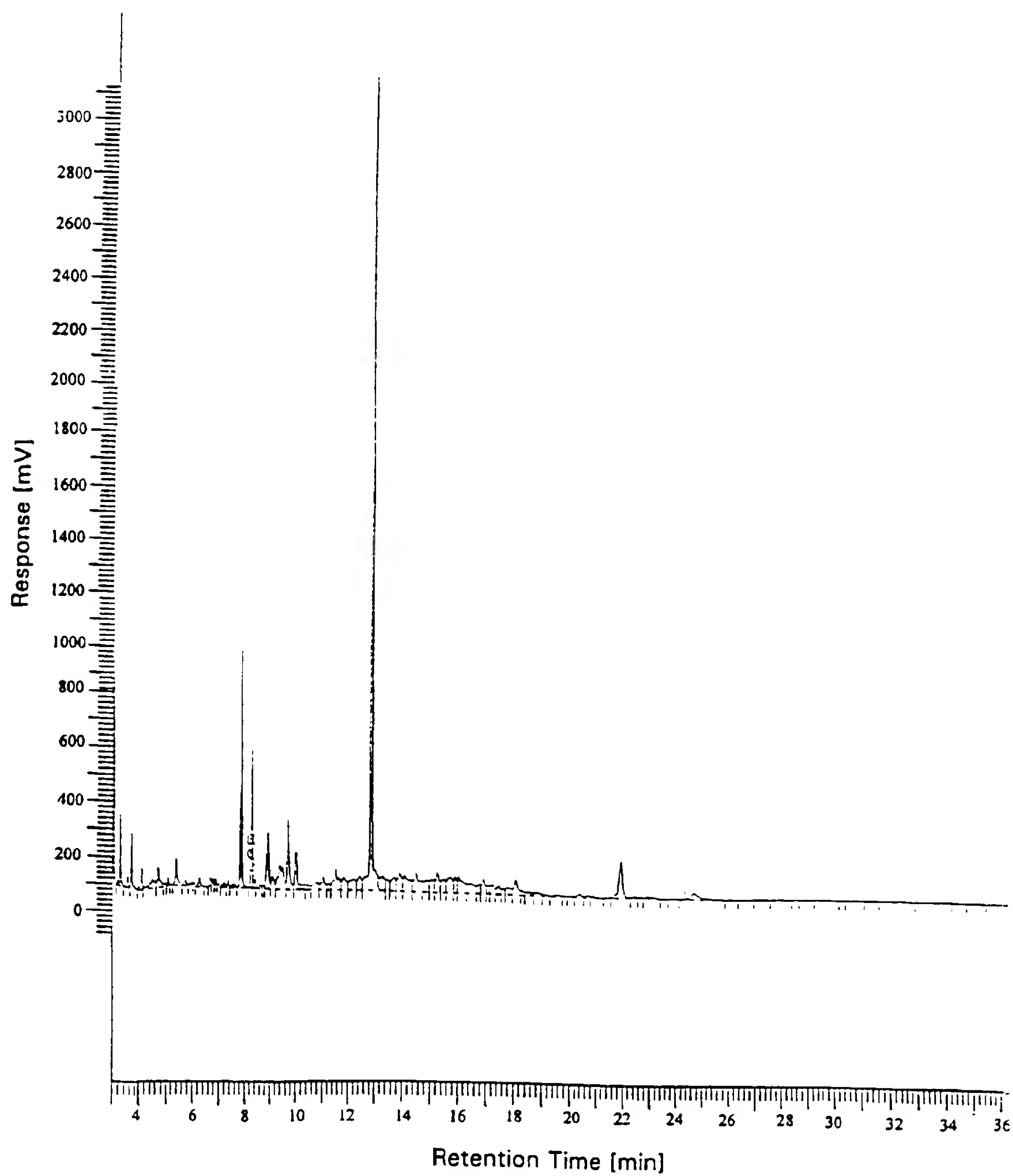
12/16

FIG. 12



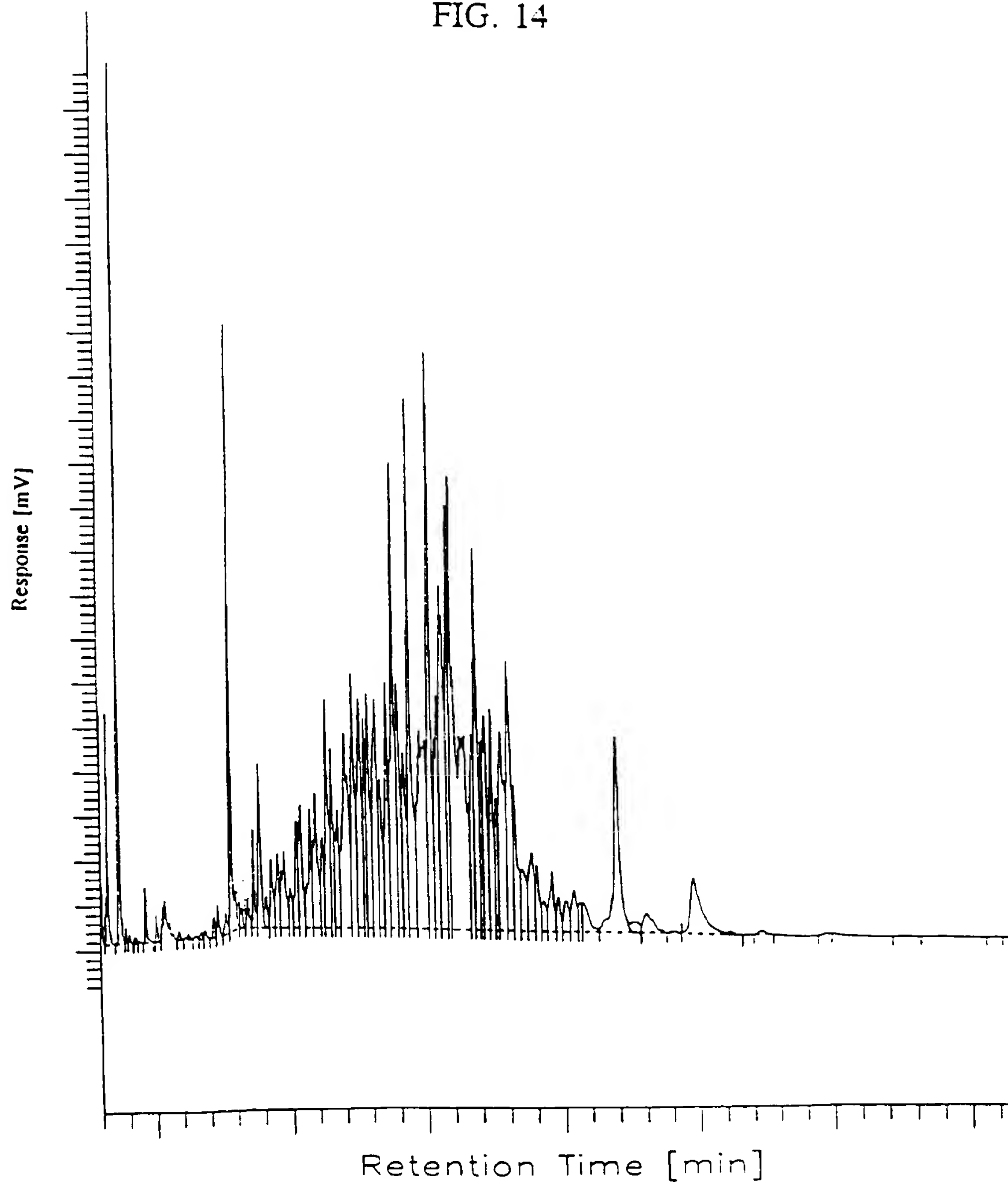
13/16

FIG. 13



14/16

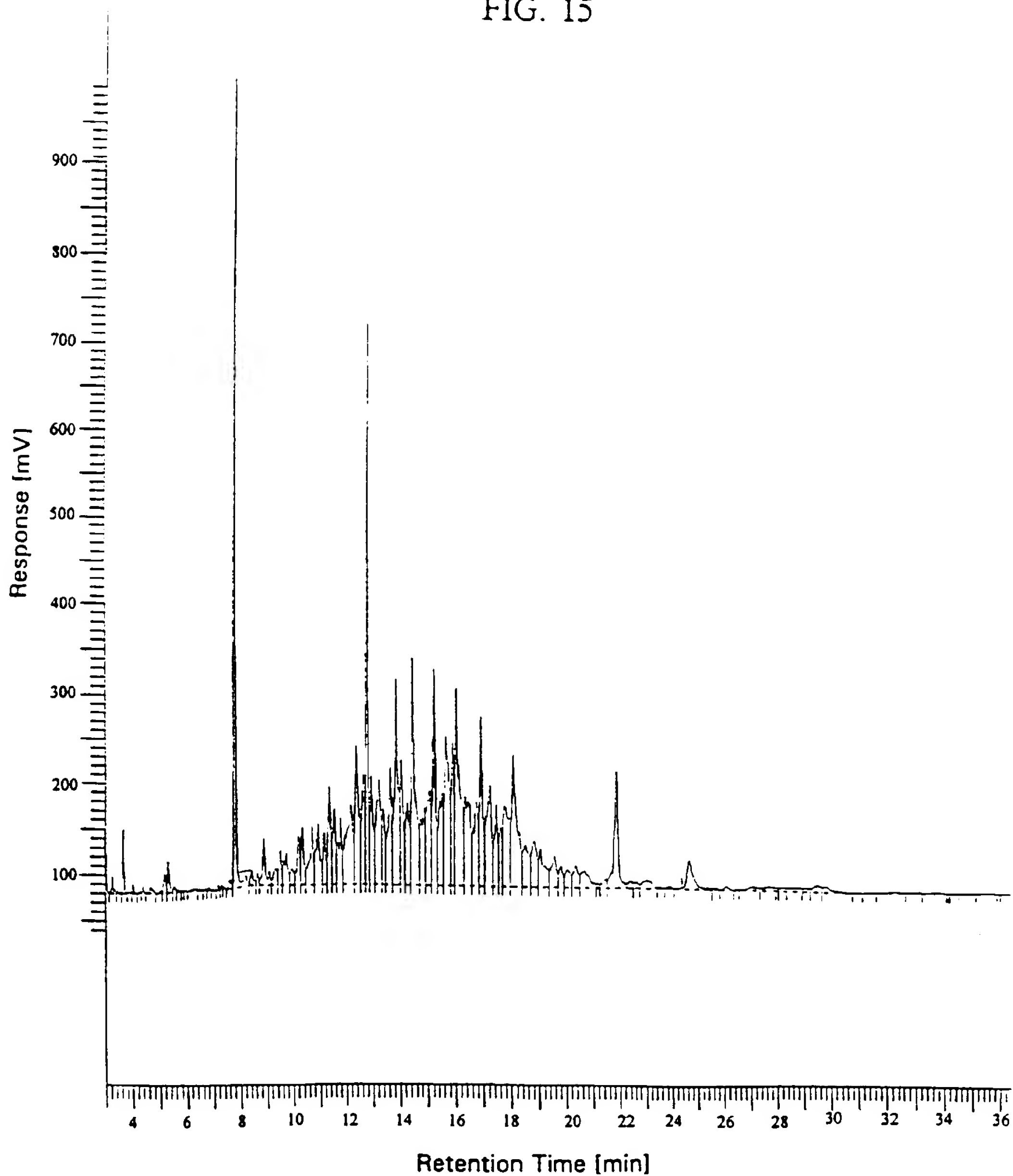
FIG. 14





15/16

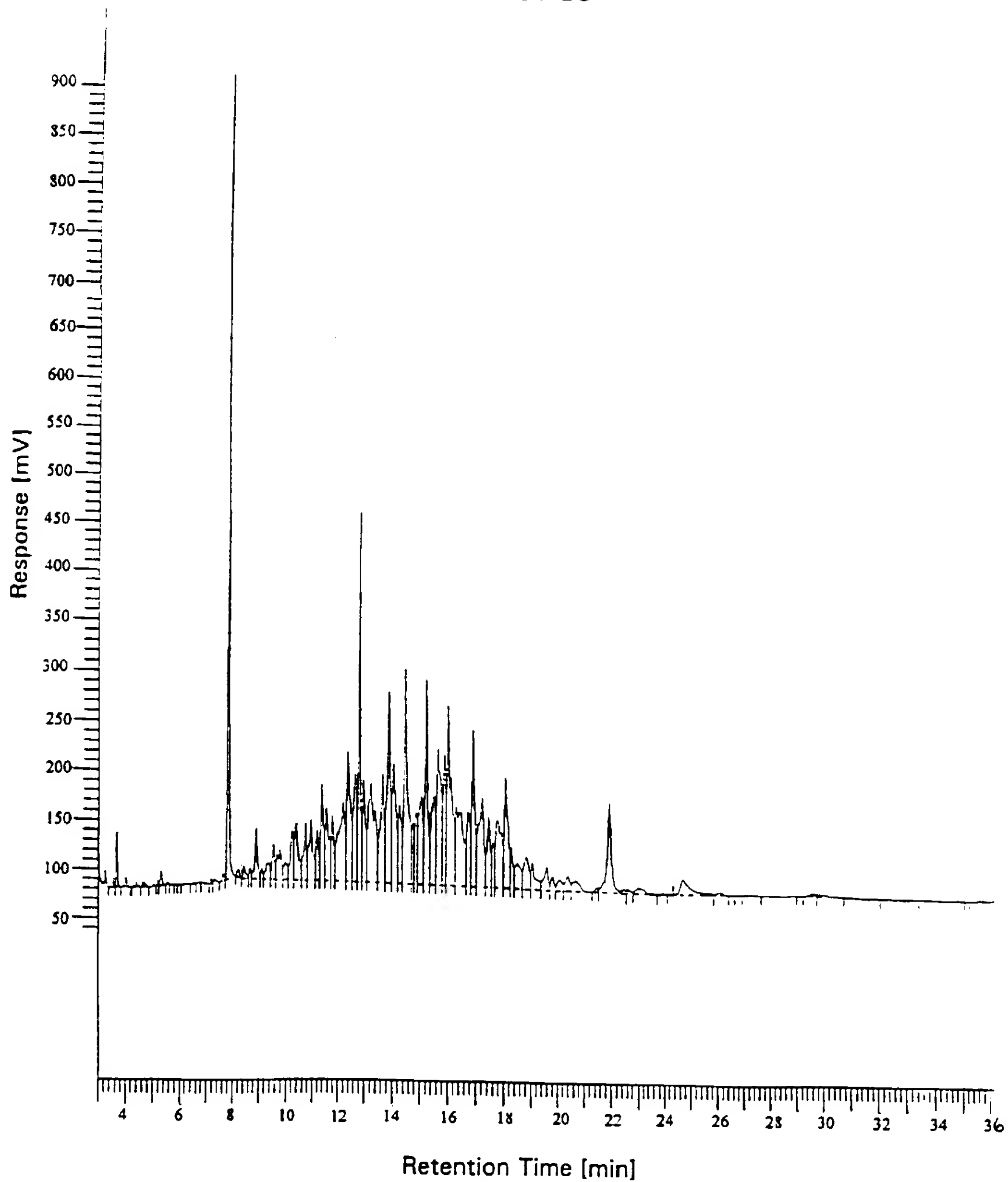
FIG. 15



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16/16

FIG. 16



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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/05532

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : B09B 3/00; B09C 1/00

US CL : 435/262, 262.5

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/262, 262.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,227,136 (HANIFY ET AL) 13 July 1993, col. 2, lines 10 to 12 and 50 to 53, col. 4, lines 6 to 11 and 41 to 46.	1-14
Y	US, A, 5,037,551 (BARKLEY ET AL) 06 August 1991, col. 3, lines 26 to 46, col. 5, lines 4 to 14, col. 8, lines 31 to 68.	1-14
Y	US, A, 4,664,805 (FOCHT) 12 May 1987, col. 4, lines 18 to 43, col. 5, lines 16, lines 47.	1-14



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

17 JULY 1996

Date of mailing of the international search report

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